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Original Communication

Significance of teeth lead accumulation in age estimation

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ABSTRACT

Lead accumulation in 398 permanent teeth of Kuwaiti subjects, aged between 11 and 74 years (mean age = 25 ± 8 years) was analyzed by atomic absorption spectrophotometry. A significant correlation was found between dentin lead levels and age. The mean dentin lead concentration was significantly higher in males than in females (6.8 ± 4.7 and 5.6 ± 4.6, respectively, $p = 0.015$). A formula was established to calculate age from lead levels in teeth (Age = 1.2 × dentin Pb + 17.6). The proposed formula was applied to a validation group of 90 Kuwaiti subjects (45 males and 45 females, aged between 13 and 58 years). The standard error of age estimation, applying the proposed formula, was ±5.8 years with R square = 52.3%. The mean difference between the true and calculated ages was 1.3 ± 4.8 years. The coefficient of variation was 43%. The proposed formula is merely applicable to Kuwaiti population, and can be of a significant value in forensic practice whenever examining human skeletal remains, particularly of Kuwaiti war victims. Other formulas may well be established via similar studies on dentin lead levels on various populations.

1. Introduction

Age estimation may pose a real difficulty in the forensic practice. Such difficulty is more evident when examining skeletal remains of adults than in children or adolescents.[1–4] Accumulation of trace elements in teeth may show correlation with age.[5] Lead is one of the most significant pollutants in the environment and has greatly attracted researchers' attention due to its toxicity to humans.[6] The concentration of lead in teeth is a cumulative meaning of earlier exposure, contrary to blood lead concentration, which is an "instantaneous" indicator that reflects immediate exposure.[7] Lead exposure can thus be shown by means of teeth lead concentration. Dentin is the main site for lead deposition and has been shown to provide evidence of early exposure until the time the tooth is extracted.[7,8] Furthermore, no significant difference has been reported between the lead levels of different tooth types.[9]

Although it is well established that lead concentration in permanent teeth increases with the advancement of age,[5,10] yet no adequate statistical analyses have been applied to verify the significance of differences in lead levels among different age groups.

The aim of this work is to verify whether lead levels in teeth are correlated with age and possibly gender, and if such correlation can be utilized for the purpose of age estimation through teeth lead levels.

2. Material and methods

A total of 398 permanent teeth (first premolar and third molar) were collected from 192 males and 206 females aged between 11 and 74 years, divided into 5 age groups and analyzed for dentin lead concentration. Teeth were collected from the Department of Oral Surgery, Kuwait Dental Center during the period from January 1999 through December 2002. The tested teeth were extracted from living Kuwaiti subjects from urban and suburban population, who were not suffering from known medical diseases. None of the donors were directly or in some way occupationally-exposed to lead contamination e.g. worked in connection with road vehicles or industrial emission, nor did they have history of other means of lead exposure. All examined teeth were free from restorations and/or dental caries and all were extracted because of periodontal disease and/or orthodontic reasons.

All glass and plastic ware were soaked in a 20% nitric acid solution for 24 h and then rinsed thoroughly with deionized water. Each extracted tooth was rinsed with distilled water then immersed in a high density polypropylene vial containing a 10% sodium hypochlorite solution to remove residual soft tissues.[11,12] The crown and the apical third parts of the root were detached. The cement was removed with a dental drill and any residual pulp tissue was carefully removed using endodontic reamers.

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Fresh dentin samples (0.1–0.5 g) were dried at 105 °C for 24 h.[5,13] Hydrolyzation of dentin was performed in special metal-free vacationer glass tubes (B & D Royal Blue). Each sample was weighed and digested by 1 mL concentrated nitric acid $M = 63.01$ g/mol (Riedel de Haea Germany UN No 2031). The samples were incubated overnight at 60 °C until the dentin was completely digested. At the following day, the clear supernatants were diluted to 25 ml with distilled and deionized water.[13] The diluted samples were preserved at −4 °C for further analysis.

The levels of dentin lead concentrations were estimated by Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) with Zeeman correction (Varian model, Spectra AA 800 GTA Australia). Prior to determination of the levels of lead, the instrument was standardized according to the operating parameters described in the manufacturer's operating manual. Stock standards were obtained from Fluka Chemika (Switzerland) and were run in the calibration range of 10.0–40.0 PPM. All samples were diluted with distilled water and contained the same acid concentration as the standards. The diluents were analyzed for any possible contamination.

The Statistical Package for the Sigma Stat 3.0 was used. A regression formula was established to calculate age from lead levels in teeth. The proposed formula was applied to a validation group consisting of 90 Kuwaiti donors of known ages (45 males and 45 females, aged between 13 and 58 years). Similar permanent teeth were extracted and analyzed for dentin lead levels by a similar methodology. The calculated ages, using the regression formula and the chronological ages, were statistically analyzed.

3. Results

Table 1 shows the dentin lead levels in various age groups amongst the 398 tested subjects. ANOVA test revealed a significant difference in dentin lead levels among the different groups in both genders ($p < 0.05$). The variance was not homogeneous and hence, post-hoc analysis was performed by Tamahne test. The mean dentin lead concentration was significantly higher in males than in females (6.8 ± 4.7 μg/g and 5.6 ± 4.6 μg/g respectively; $p = 0.015$).

Fig. 1 illustrates the linear relationship between age and dentin lead levels in the test group ($R^2 = 52.3$). The correlation coefficient for dentin lead levels and age in the test group was 0.654 ($p < 0.0001$). The standard error of regression was 5.95 years. The coefficient of variation was 43%, in the entire test group, but when gender was taken into consideration, it was 51% in females and 41% in males.

The linear regression formula calculated for dentin lead concentration and age in the test group was:

$$y = 1.2x + 17.6$$

(y = subject's age and x = dentine lead concentration).

When the regression formula was applied to the validation group (45 males and 45 females aged between 13 and 58 years with a mean age 26 ± 10 years), the mean difference between the true age and calculated age (using the regression formula) was 1.3 ± 4.8 years. Figs. 2 and 3 show the variation between the true ages and the calculated ages, after the regression formula was employed, on the validation group. Table 2 compares the true and calculated ages among subgroups of the validation sample. It has demonstrated (as also shown in Figs. 2 and 3) overestimation of the calculated ages among the younger age subgroups (below 20 years) in both genders, which was statistically significant ($p < 0.001$). On the other hand, the male older age subgroup (above 30 years) demonstrated underestimation of the calculated ages, which was statistically significant ($p \leq 0.05$). However in the subgroups between 20 and 30 years of both genders, which constituted the largest number of the validation sample (51 out of 90 subjects), there was no statistically significant difference between the true and the calculated age when the proposed formula was employed.

Table 1
Dentin lead levels amongst various test groups (total # = 398 subjects).

Age Groups	N	$\underline{X}$	SE	Min–Max
<20	79	3.9495	0.32634	0.80–14.90
21–30	232	4.9675	0.15662	1.10–17.54
31–40	62	9.6995	0.68737	1.20–25.21
41–50	17	17.2347	1.46874	5.21–27.13
>50	7	15.2857	2.77741	4.30–27.13
Total	398	6.2373	0.23781	0.80–27.13

N = number of subjects, $\underline{X}$ = mean dentin lead level (μg/g), SE = standard error, Min-Max = minimum and maximum dentin levels

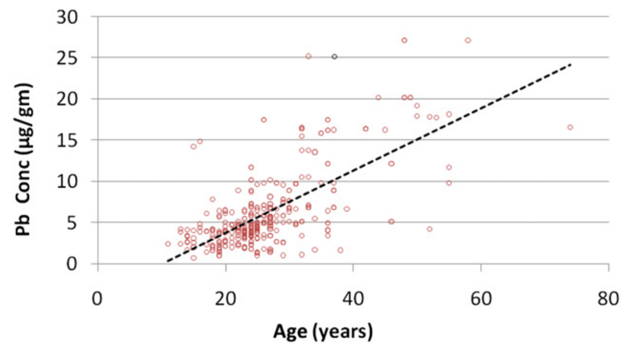

Fig. 1. Relationship between age and dentin lead concentration in the test group ($N = 398$).

4. Discussion

Lead is accumulated in bones and teeth,[14] but the amount of lead released from teeth is insignificant.[15] Its annual aggregation in teeth can be considered as directly correlated to blood levels. Thus, teeth are good indicators of environmental lead exposure and have been used as such by some researchers.[13–17] The accumulation of lead with age, therefore, has been primarily employed as a marker of pollution rather than a means of estimating the age.

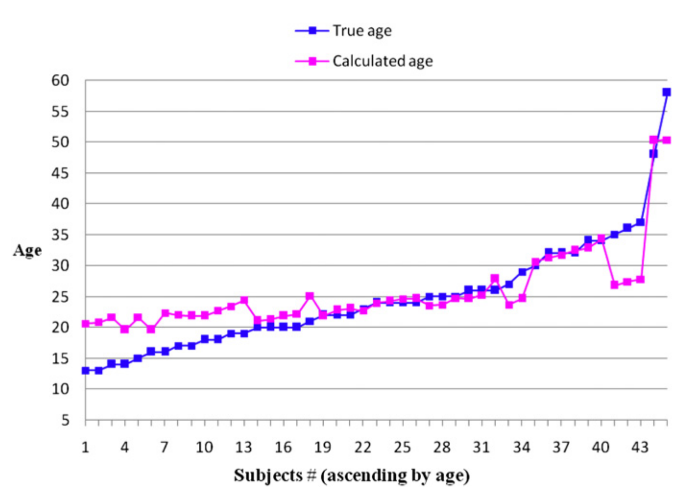

Fig. 2. Comparison of the true and calculated age for the male validation group (45 subjects).

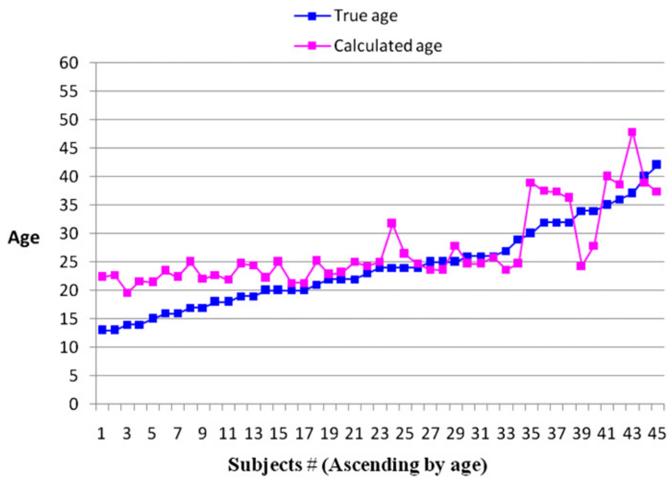

Fig. 3. Comparison of the true and calculated age for the female validation group (45 subjects).

The current study has demonstrated a significant correlation between age and dentin lead levels in a non-occupationally exposed Kuwaiti population. Statistical analyses of our data have confirmed such relationship. This is in accordance with previous reports.[5,7,10] Analogous correlation between age and dentin lead levels has also been established by Khandekar et al.,[18] although the range of each age group was 15 years and the results were not checked for significance. The previously mentioned studies, however, have not employed the relationship between age and dentin lead levels in age estimation. On the other hand, several additional studies, have reported no age association with teeth lead levels.[6,19,20,21]

It is well known that lead uptake can be variable depending upon many factors such as the proximity of exposed subjects to industrial complexes releasing large quantities of lead into the atmosphere, concentration of lead in the drinking water and fumes from car exhausts where leaded petrol is still in use.[6] Although the subjects tested in this series were not occupationally exposed to lead, Fig. 1 has demonstrated a very small number of "outlying values", particularly above the age of 20 years, with markedly elevated dentin lead levels. Such higher levels might be explained by the possibility of exposure to unidentified sources of lead contamination, such as drinking water supplied through old distributing systems (at home or at work), or through water-coolers which are quite popular in public places and were found to pose some threats in Kuwait.[22] Other possible sources of lead contamination include frequent contaminated food consumption in the open atmosphere,[19] and exposure to residential paint in older buildings.[23]

Table 3
Comparative studies on dentin lead levels.

Country	Mean age (years)[a]	Mean lead (μg/g)	No. of teeth	Reference
Kuwait	3–74	2.35	216	Bu-Olayan and Thomas, 1999[19]
Bahrain	5–15	4.3	280	Al-Mahroos and Al-Saleh, 1997[24]
Turkey	9.4	3.42	103	Karakaya et al., 1996[25]
Israel				
(Jerusalem)	20.5	1.46	143	Bercovitz et al., 1993[17]
(Haifa)	23.8	2.26		
England	6	4.00	402	Pocock et al, 1987[26]
Taiwan	8.3	4.30	390	Rabinowitz et al., 1991[27]
Italy	7	6.05	115	Bergomi et al., 1989[28]
Germany	9.4	6.16	115	Winneke et al., 1983[29]
Australia	6.8	8.60	262	McMichael et al., 1994[30]
Kuwait	25	6.24	398	Present study

[a] Range of age is given whenever the mean age was unavailable.

The mean lead level in the current series (6.24 μg/g) is much higher than previous reported figures from Kuwait in the late 90s (2.21–2.50 μg/g).[19] This is even though our series was carried out on urban and suburban population aged between 11 and 74 years, whereas the previous study included industrial and suburban population aged between 3 and 74 years. Nevertheless, our findings point out that exposure to lead is in increase in Kuwait, despite the efforts made by the local authorities to minimize such a risk, including introduction of deleaded petrol in 1989. The mean lead level in our series is also higher when compared with figures reported from other neighboring countries,[17,24,25] but within the range of values reported by several other workers (Table 3). However, it must be taken into consideration that most of the previously mentioned studies,[24–30] were carried out on much younger age groups than those of our series.

Another factor that might affect lead accumulation in humans is gender. Lead levels in lungs, salivary calculus and dense bones have been found to be higher in males than in females.[31–33] Both genders, if not occupationally-exposed, are presumably subject to the same environment pollution conditions. Moreover, there is no sex dissimilarity between the anatomy of the gastrointestinal tract, or the lungs. Hence, lead absorption and accumulation in human tissues should be alike in both males and females. However, the results of this study have demonstrated significantly higher levels of dentine lead in Kuwaiti males compared with females. Previous studies have published similar findings.[7,33,34] Nonetheless, other investigators have reported no significant variation between dentin lead levels of males and females.[5,6,18,19] Although the work of Steenhout and Pourtois[7] has shown significant higher dentin lead levels in males, such observation was attributed to their occupation, since they worked closer to industrial areas. Our findings indicate

Table 2
Comparison between true and calculated age among subgroups of the validation sample (45 males and 45 females).

Gender	Subgroups (years)	Mean		SD		T (*P*-value)	Significance
		True age	Calculated age	True age	Calculated age		
Male	<20	16.8	21.5	2.3	1.0	6.28	0.0001
	20–30	24.2	23.9	2.5	1.9	0.85	X[a]
	>30	41.4	35.2	11.4	13.7	2.80	0.05
Female	<20	16.1	22.7	2.1	1.5	13.4	0.0001
	20–30	23.6	24.6	2.6	2.3	1.8	X[a]
	>30	34.9	36.8	3.6	6.2	0.98	X[a]

Number of male subgroups (<20 = 13; 20–30 = 21; > 30 = 11).
Number of female subgroups (<20 = 10; 20–30 = 30; > 30 = 5).
[a] X = not significant.

that gender can effectively influence lead accumulation in human teeth. Therefore, this must be taken into consideration when comparing lead exposure of different populations.

The present work has proposed a seemingly reliable formula, which can be employed for estimating the age from dentin lead levels in Kuwaiti population. When the proposed formula was applied to a validation group of both genders, comparison of the true age and the calculated age utilizing that regression formula showed no statistical significant difference for the entire male and female groups ($p = 0.06$ and 0.932 respectively). Nevertheless, our results have demonstrated some overestimation of the calculated age when the proposed formula was applied to subjects below the age of 20 years in both genders and a few number of underestimations in males above the age of 30 years, as shown in Figs. 1 and 2 and Table 2. Such a formula can be extremely useful in forensic investigations; particularly those involve examination of skeletal remains and specially those of missing Kuwaiti war victims. However, it must be emphasized that the proposed formula is only applicable to Kuwaiti population. Further research is required in order to find out whether similar formulas can be implemented on other populations.

Conflict of interest

The authors of this submitted manuscript hereby declare that there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three (3) years of beginning this work.

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